brain of the hypertensive patients. However, in the present study, oxidative stress in the RVLM is significantly reduced and baroreflex sensitivity is significantly improved by atorvastatin, whereas BP and sympathetic nerve activity are not altered. These results suggest that oxidative stress is inhibited and baroreflex sensitivity is improved by atorvastatin whose dose is insufficient for the reduction of blood pressure or sympathetic nerve activity. The improvement of baroreflex sensitivity could not be explained by the effect of atorvastatin on peripheral mechanisms, and we consider that baroreflex sensitivity is improved by the central action of atorvastatin. Clinical studies suggest that clinical doses of statins have the beneficial effect on arrhythmic sudden death and ventricular arrhythmia in the patients with heart failure, and these effects may be due to the improvement of the imbalance between sympathetic and parasympathetic nerve activity (18). It is necessary to examine the effect of clinical doses of atorvastatin on baroreflex sensitivity in a clinical study.

There are some limitations in the present study. First, we measured TBARS levels as the parameter of oxidative stress in the brain. Thiobarbituric acid-reactive substance levels are an indirect marker of oxidative stress, and there are other methods to measure oxidative stress. However, we previously measured oxidative stress directly in the brain of SHRSP and WKY using electron spin resonance spectroscopy and confirmed that TBARS levels are comparable to the levels of oxidative stress measured by electron spin resonance spectroscopy in the brain (4). The results suggest that TBARS levels are a valid parameter of oxidative stress in the brain. Second, we did not examine the TBARS levels in other areas of the brain, such as caudal ventrolateral medulla, nucleus tractus solitarii, paraventricular nucleus, cortex, hypothalamus, and cerebellum. We consider that these effects of atorvastatin was not unique in the RVLM, and we did not exclude the possibility that atorvastatin influences those areas thereby improving baroreflex control of HR in the present study. However, RVLM is the vasomotor center, and the integrated various inputs from other regions to RVLM influence the sympathetic outflow (1–3). Although it would be interesting to examine these parameters in other regions of the brain, we targeted the changes of oxidative stress in the RVLM due to atorvastatin in the present study.

Conclusions

Our results suggest that oral administration of atorvastatin improved the baroreflex control of heart rate due to the inhibition of oxidative stress in the RVLM of SHRSP.

Acknowledgments

We are grateful to Pfizer. USA, for supplying atorvastatin. This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (B19390231), and in part, by the Health and Labor Sciences Research Grant for Comprehensive Research in Aging and Health Labor and Welfare of Japan.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Bionic Autonomic Neuromodulation Revolutionizes Cardiology in the 21st Century

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Abstract — **In this invited session, we would like to address the impact of bionic neuromodulation on cardiovascular diseases. It has been well established that cardiovascular dysregulation plays major roles in the pathogenesis of cardiovascular diseases. This is the reason why most drugs currently used in cardiology have significant pharmacological effects on the cardiovascular regulatory system. Since the ultimate center for cardiovascular regulation is the brainstem, it is conceivable that autonomic neuromodulation would have significant impacts on cardiovascular diseases. On the basis of this framework, we first developed a bionic, neurally regulated artificial pacemaker. We then substituted the brainstem by CPU and developed a bionic artificial baroreflex system. We further developed a bionic brain that achieved better regulatory conditions than the native brainstem in order to improve survival in animal model with heart failure. We recently developed a bionic neuromodulation system to reduce infarction size following acute myocardial infarction. We believe that the bionic neuromodulation will inspire even more intricate applications in cardiology in the 21st century.**

I. OVERVIEW OF PREVIOUIS BIONIC STUDIES

In the human body, all cells, tissues, organs, and systems operate coherently. The presence of well-developed neurohormonal communications among these components of the body is the essential infrastructure that makes coherent functioning possible. If we could incorporate such communication mechanisms into artificial systems, they would function as if they are an integral part of the corresponding native physiological systems. We call such well-integrated artificial systems bionic systems.

The bread-and-butter technology that is common to all bionic systems is the technique for interfacing with the native systems, in particular, the human body's regulatory systems. Unification of an artificial system with a native system requires bidirectional communications. In 1995, we developed one such system, a neurally regulated artificial pacemaker [1]. Physiological studies indicated that the instantaneous sinus rate was determined not only by the current sympathetic activity but also by the history of sympathetic activity. We quantified its history dependence by the impulse response of the sinus rate to sympathetic stimulation. Using the convolution integral of the impulse response with the instantaneous sympathetic activity, we could predict the precise sinus rate in real time [1].

The success of the neurally regulated bionic pacemaker has convinced us that the autonomic system can be effectively monitored and thereby manipulated by bionic systems. The clinical impact of direct manipulation of autonomic functions in cardiovascular diseases is very profound. The case of central baroreflex failure is an archetypal example of one such application. In treating this disease, it is conceivable that one can implement an artificial bionic baroreflex system as a kind of biological proxy capable of emulating the native central baroreflex function of the failing vasomotor center. The bionic baroreflex system consists of a pressure sensor (baroreceptor), microprocessor (vasomotor center) and nerve stimulator (for activation of sympathetic efferents). The system operates as an intelligent negative feedback regulator, and has been demonstrated in animals and patients to be effective in restoring normal baroreflex functioning [2-5].

Recently, we developed an artificial brain stem that takes over the native cardiac regulation, and optimized it to improve the survival of chronic heart failure [6]. Two weeks after the ligation of the left coronary artery in rats, surviving animals were randomized to vagal- and sham-stimulated groups. Vagal stimulation markedly improved the 140-day survival (86% versus 50%, P=0.008). The relative risk reduction of death reached over 70%. The success of the bionic treatment of heart failure opens up an entirely new therapeutic paradigm for patients with chronic heart failure.

II. BIONIC NEUROMODULATION IN ISCHEMIA

Although the bionic autonomic neuromodulation system prevented progression, thereby improved survival of chronic heart failure, it would be far desirable if we can prevent the development of heart failure. Ischemic heart disease has been known as one of the major causes of heart failure. Therefore, we examined whether bionic autonomic neuromodulation impacts ischemia-reperfusion injury of the heart. This particular application of bionic autonomic neuromodulation is critically important under clinical settings because early reperfusion of occluded coronary arteries has become a standard therapy worldwide.

Myocardial infarction has been known to augment sympathetic afferent traffic and reduce vagal efferent activity. We investigated whether short-term electrical stimulation of

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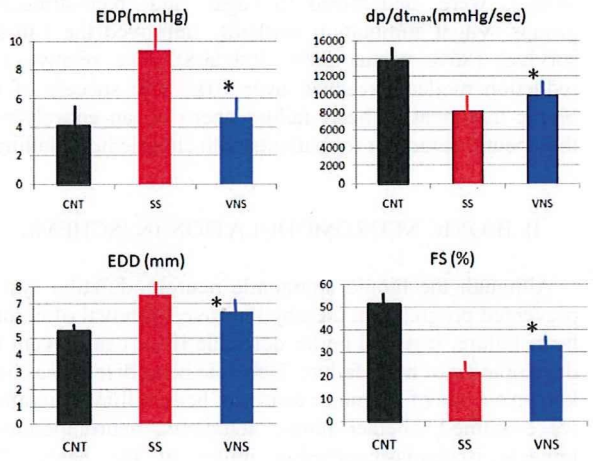
the vagal nerve could ameliorate cardiac dysfunction in a distant period after ischemia-reperfusion injury.

Ischemia-reperfusion injury model was created in Sprague-Dawley rats by ligating the left coronary artery for 30 min followed by reperfusion. We stimulated the right vagal nerve (the stimulation condition is proprietary) from the onset of ischemia for 3 hrs. We measured hemodynamics before ischemia, 4 days after ischemia with and without bionic autonomic neuromodulation. We estimated left ventricular function using echocardiography. We estimated infarction size histologically 4 days after ischemia.

III. RESULTS

As shown in the upper panels of figure, in comparison with sham stimulation (SS, n=6), vagal nerve stimulation (VNS, n=6) significantly decreased left ventricular end-diastolic pressure, and increased left ventricular (dp/dt)max suggesting improved left ventricular function. CNT represents the control condition (n=4). The improvement of left ventricular function was paralleled with decreased end-diastolic dimension (EDD), and increased shortening fraction (EF) as shown in the lower panels in the figure. Histological examination further supported the notion that vagal stimulation decreased the infarction size (33±5% vs. 24±3%, p<.01). Biochemical analysis indicated that vagal stimulation downregulated mRNA of procollagens, such as Col1a1, Col3a1, and Ctgf, in infarcted myocardium. Therefore, the positive impact of vagal nerve stimulation might have, at least in part, resulted from inhibition of collagen production in ischemia-reperfusion injury.



IV. DISCUSSION

We have shown that vagal stimulation early after the creation of ischemia resulted in marked reduction in infarction size and improvement of left ventricular function with attenuated cardiac remodeling. Although the effect of vagal nerve stimulation on long term survival remains to be investigated, it is conceivable that the vagal nerve stimulation early after ischemia-reperfusion injury may have a positive impact on such a hard endpoint.

The mechanism by which the bionic neuromodulation improves ischemia-reperfusion injury remains unknown. The bradycardiac effect of vagal stimulation might be a contributing factor. However, our pilot study indicated that a comparable heart rate reduction induced by beta-blocker failed to show the positive impacts on ischemia-reperfusion injury as much as the vagal stimulation did. Theretofore, mechanisms other than the bradycardiac effect such as energy sparing effect, anti-inflammatory effect and anti-oxidant effect need to be considered [7-10].

V. CONCLUSION

Vagal nerve stimulation reduces infarct size, improves left ventricular function and attenuates left ventricular remodeling after ischemia-reperfusion injury. Bionic autonomic neuromodulation should inspire even more intricate applications in cardiology in the 21st century.

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Inhibition of Prolyl Hydroxylase Domain-Containing Protein Suppressed Lipopolysaccharide-Induced TNF-α Expression

Kotaro Takeda, Toshihiro Ichiki, Eriko Narabayashi, Keita Inanaga, Ryohei Miyazaki, Toru Hashimoto, Hirohide Matsuura, Jiro Ikeda, Toshio Miyata, Kenji Sunagawa

Objective—Prolyl hydroxylase domain-containing proteins (PHDs) play pivotal roles in oxygen-sensing system through the regulation of α-subunit of hypoxia-inducible factor (HIF), a key transcription factor governing a large set of gene expression to adapt hypoxia. Although tissue hypoxia plays an essential role in maintaining inflammation, the role of PHDs in the inflammatory responses has not been clearly determined. Here, we investigated the role of PHDs in lipopolysaccharide (LPS)-induced tumor necrosis factor α (TNF-α) induction in macrophages.

Methods and Results—Northern blot analysis and ELISA revealed that LPS-induced TNF-α upregulation was strongly suppressed by PHD inhibitors, dimethyloxallyl glycine (DMOG), and TM6008 in RAW264.7 macrophages. DMOG suppressed LPS-induced TNF-α upregulation in HIF-1α–depleted cells and HIF-1α overexpression failed to suppress the induction of TNF-α. DMOG rather suppressed LPS-induced NF-κB transcriptional activity. Downregulation of *Phd1* or *Phd2* mRNA by RNA interference partially attenuated LPS-induced TNF-α induction. DMOG also inhibited LPS-induced TNF-α production in peritoneal macrophages as well as human macrophages.

Conclusions—PHD inhibition by DMOG or RNA interference inhibited LPS-induced TNF-α upregulation in macrophages possibly through NF-κB inhibition, which is independent of HIF-1α accumulation. This study suggests that PHDs are positive regulators of LPS-induced inflammatory process, and therefore inhibition of PHD may be a novel strategy for the treatment of inflammatory diseases. (***Arterioscler Thromb Vasc Biol.* 2009;29:2132-2137.)**

Key Words: tumor necrosis factor -alpha ■ prolyl hydroxylase domain-containing protein ■ hypoxia-inducible factor ■ inflammation ■ hypoxia

Inflammation is a fundamental process for the protection of our body against outside pathogen. Tissues with inflammation are characterized by several features including the accumulation of inflammatory cells such as macrophages, lymphocytes, and neutrophils, limited blood supply attributable to impaired local microcirculation, and abnormal angiogenesis.[1] Inflammatory cells are metabolically active and consume a large amount of oxygen and nutrient. These cells are, therefore, eventually exposed to hypoxic and nutrient-deprived condition.[2] Thus, the inflammatory cells need to adapt these hypoxic conditions to perpetuate inflammatory reaction.[3]

The reduced oxygen concentration is directly sensed by an innate oxygen-sensing system.[4–6] The hypoxia-inducible factor (HIF) is a key transcription factor that mediates cellular adaptive responses to hypoxia.[7] HIF is a heterodimer consisting of an oxygen-labile α-subunit and a stable β-subunit. The stability of the α-subunit of HIF-1 and HIF-2 (HIF-1α and HIF-2α) is regulated through the hydroxylation at the 4-position of specific proline residues in HIF-1α and HIF-2α by prolyl hydroxylase domain-containing proteins (PHDs).[8,9] Because PHD activity depends on the availability of molecular oxygen, PHDs are able to serve as a sensor for oxygen concentration. Under normal oxygen concentration, HIF-α is well hydroxylated by PHDs and tagged by von Hipple-Lindau (VHL) E3 ubiquitin ligase complex to be targeted for proteosomal degradation.[8,9] When oxygen concentration is reduced, the activity of PHDs is decreased. This results in the accumulation of HIF in the nucleus, followed by upregulation of a series of genes suited for hypoxic condition.

Because hypoxia is closely associated with an inflammatory reaction, it is reasonable that HIF is essential to maintain inflammatory processes. By switching energy production from oxidative phosphorylation to an anaerobic metabolism, macrophages generate ATP and thereby preserve its bactericidal ability in the hypoxic tissues.[10,11] HIF-1α–deficient myeloid cells showed impaired inflammatory responses attributable to inefficient energy production.[10,12] In contrast to HIF, the role of PHD in the inflammation is somewhat controversial. A specific knockdown of *Phd* gene led to the activation of NF-κB and hence upregulation of proinflamma-

Received May 17, 2009; revision accepted September 3, 2009.

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Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org DOI: 10.1161/ATVBAHA.109.196071

tory molecules in HeLa cells.[13] On the other hand, chemical PHD inhibitors attenuated inflammatory responses in several models including colitis and myocardial inflammation after an ischemic insult.[14–16] Thus, in the present study, we focused on the question whether PHD inhibition suppresses or activates inflammatory responses in macrophages. We demonstrated that the PHD inhibition by pharmacological inhibitors or RNA interference suppressed lipopolysaccharide (LPS)-elicited induction of tumor necrosis factor α (TNF-α),[17] a pivotal proinflammatory cytokine. However, interestingly the suppression was mediated not by a HIF-α accumulation but by suppression of NF-κB transcriptional activity. Our data suggest that suppression of PHD may be a novel antiinflammatory mechanism.

Methods

To clarify the role of PHD inhibition on inflammatory response, murine macrophage cell line, RAW264.7 cells were stimulated with LPS in the presence or absence of PHD inhibitor. The effect of LPS on mouse peritoneal macrophage, and human monoctye cell line, THP-1 was also examined. PHD isoforms were selectively knocked down by stable transfection of small hairpin RNA expression vector. Expression of TNF-α and other inflammatory cytokines were examined by quantitative reverse-transcription PCR (qPCR) or Northern blot analysis. Promoter activity was examined by luciferase assay. Nuclear translocation of NF-κB was examined by electrophoretic mobility shift assay and ELISA-based TransAM NF-κB p65 Transcription Factor Assay Kits. Cell viability was measured by flow cytometry after propidium iodide statining.

Detailed information of materials and methods used in this article is available in the online Data Supplement (please see http://atvb.ahajournals.org).

Results

DMOG Suppressed LPS-Induced TNF-α Upregulation in Macrophages

To assess the effect of the PHD inhibition on inflammatory response, RAW264.7 macrophages were pretreated with a vehicle DMSO or DMOG (1 mmol/L) for 1 hour before 100 ng/mL of LPS stimulation. Real-time qPCR and Northern blot analysis revealed that DMOG time- and dose-dependently inhibited LPS-induced *Tnf-α* mRNA upregulation (Figure 1A and 1B and supplemental Figure IA and IB). TNF-α secretion in the supernatant during 24 hours of LPS treatment was also suppressed by DMOG (Figure 1C).

A luciferase gene regulated by murine *Tnf-α* gene promoter was introduced into the RAW264.7 cells, and luciferase activity was measured. A LPS treatment (100 ng/mL for 6 hours) significantly increased *Tnf-α* promoter activity and DMOG significantly suppressed the upregulation (Figure 1D). In contrast, DMOG did not affect *Tnf-α* mRNA stability (data not shown). We tested another novel PHD inhibitor, TM6008.[18] Pretreatment with TM6008 (100 μmol/L) for 1 hour significantly suppressed TNF-α secretion in the supernatant after 24 hours of LPS treatment (supplemental Figure II). In addition to TNF-α, DMOG suppressed LPS-induced TNF-α converting enzyme (*Tace*) expression (supplemental Figure III).

Phd Knockdown Strongly Attenuated the LPS-Induced Cytokine Production

To examine whether the suppressive effect of DMOG is indeed mediated by the PHD inhibition, *Phd* gene expression

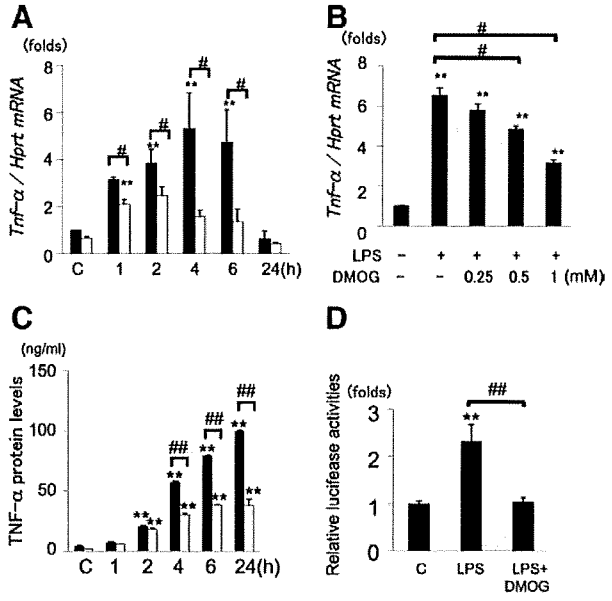

Figure 1. DMOG suppressed LPS-induced TNF-α upregulation in RAW264.7 macrophages. A, After pretreatment with 1 mmol/L of DMOG (open bar) or a vehicle DMSO (filled bar) for 1 hour, RAW264.7 cells were stimulated with LPS (100 ng/mL) for varying periods indicated in the figure. *Tnf-α* mRNA was determined by real-time qPCR. B, The effect of varying concentrations of DMOG pretreatment for 1 hour on LPS (100 ng/mL, 4 hours)-induced *Tnf-α* mRNA expression was examined. *Tnf-α* mRNA level was normalized with the level of *Hprt* mRNA. C, TNF-α concentration in the supernatant of RAW264.7 cells during 24 hours of LPS (100 ng/mL) treatment with the pretreatment of 1 mmol/L of DMOG (open bar) or a vehicle DMSO (filled bar) for 1 hour was determined by ELISA. D, LPS-induced *Tnf-α* gene promoter activity after 100 ng/mL of LPS treatment for 6 hours with pretreatment of 1 mmol/L of DMOG or DMSO for 1 hour was measured as luciferase activity. n=3 to 4. $^{**}P<0.01$ vs control, $\#P<0.05$, $\#\#P<0.01$ vs LPS (alone).

was knocked down by shRNA introduction. Because there are at least three PHD isoforms (PHD1, PHD2, and PHD3) in mice,[19] we determined the expression of *Phd* isoforms in RAW264.7 macrophages. Real-time qPCR analyses revealed that *Phd3* gene was expressed at very low level in RAW264.7 cells (Figure 2A). We, therefore, downregulated *Phd1* and *Phd2* expression by shRNA. *Phd1* and *Phd2* shRNA efficiently decreased *Phd1* and *Phd2* mRNA expression by 91±1% and 67±2%, respectively (Figure 2B). Although *Phd2* shRNA did not affect *Phd1* mRNA expression, *Phd1* shRNA increased *Phd2* mRNA expression by 1.4-fold (Figure 2B). Then, these *Phd1*- or *Phd2*-depleted cells were stimulated with 100 ng/mL of LPS. LPS-induced *Tnf-α* mRNA upregulation and TNF-α secretion were significantly inhibited in both *Phd1*- and *Phd2*-depleted cells (Figure 2C and 2D and Figure IV). However, *Phd1* depletion showed stronger suppression of *Tnf-α* expression than *Phd2* depletion.

Activation of HIF Pathway by DMOG or *Phd2* Knockdown but not by *Phd1* Knockdown

To confirm whether the DMOG inhibition of PHD activates the HIF pathway in RAW264.7 macrophages, the levels of 2 main HIF-α isoforms (HIF-1α and HIF-2α) were determined

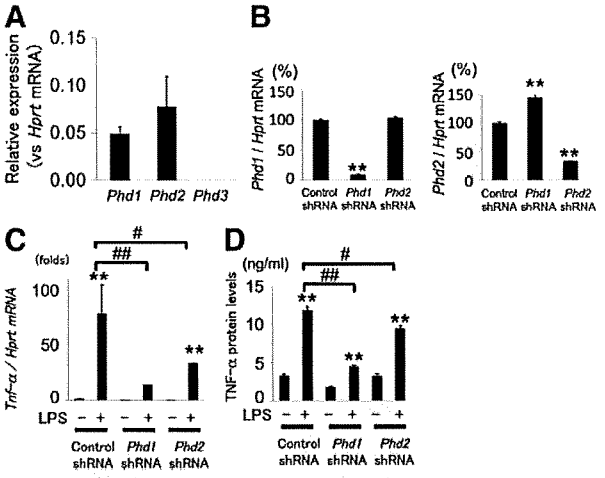

Figure 2. *Phd* knockdown suppressed LPS-induced TNF-α upregulation in RAW264.7 macrophages. A, The expression of *Phd1–3* mRNA was analyzed by real-time qPCR. B, The expression of *Phd1* or *Phd2* mRNA in control or *Phd1*- or *Phd2*-specific shRNA expressing cells was analyzed by real-time qPCR. C, Real-time qPCR analysis for *Tnf-α* mRNA in *Phd1*- or *Phd2*-depleted cells with or without LPS stimulation (100 ng/mL, 4 hours). D, ELISA for TNF-α concentration in the supernatant was performed in *Phd1*- or *Phd2*-depleted cells with or without LPS stimulation (100 ng/mL, 24 hours). n=3 to 4. #P<0.05, ##P<0.01 vs LPS (alone), **P<0.01 vs control or LPS (−).

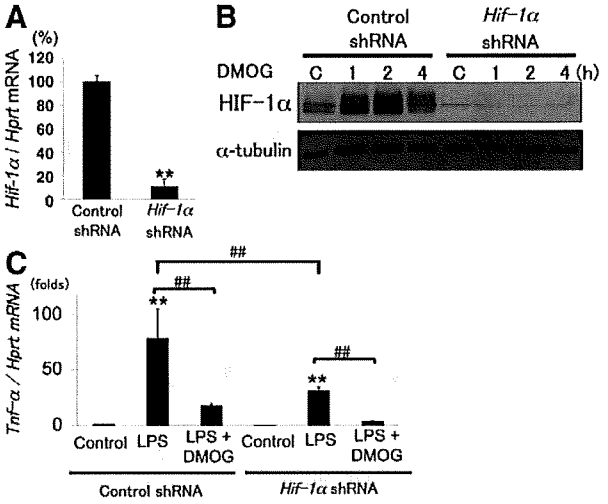

Figure 4. DMOG suppressed LPS-induced *Tnf-α* upregulation in HIF-1α–depleted macrophages. A, *Hif-1α* mRNA expression was determined by real-time qPCR. B, Western blot analysis for HIF-1α in control or *Hif-1α* shRNA expressing RAW264.7 cells after 1 mmol/L of DMOG treatment for varying periods indicated in the figure. The same results were obtained in other 2 independent experiments. C, LPS (100 ng/mL, 4 hours)-stimulated *Tnf-α* expression in control or *Hif-1α* shRNA expressing cells with pretreatment of 1 mmol/L of DMOG or a vehicle DMSO for 1 hour was determined by real-time qPCR. n=3 to 4. **P<0.01 vs control, ##P<0.01.

by Western blot analyses. Whereas HIF-1α was dramatically accumulated by DMOG treatment, HIF-2α protein remained undetectable (Figure 3A). Western blot for HIF-2α was validated by clear detection of HIF-2α expression in placenta lysate as a positive control.[20] A HRE-driven luciferase

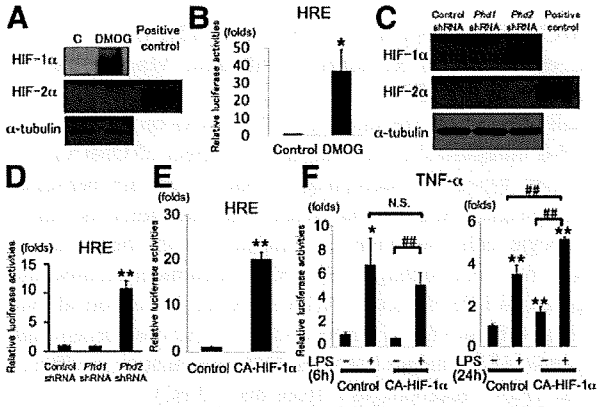

Figure 3. Expression of CA-HIF-1α failed to suppress LPS-induced *Tnf-α* upregulation. A and C, Western blot analysis for HIF-1α and HIF-2α after DMOG treatment (1 mmol/L, 6 hours) in RAW264.7 cells (A) or in *Phd1* or *Phd2* shRNA expressing RAW264.7 cells (C). The same results were obtained in 2 other independent experiments. Murine placenta total lysate was used as a positive control for HIF-2α. B and D, HRE-luciferase activities were measured in RAW264.7 cells with 1 mmol/L of DMOG or a vehicle DMSO for 24 hours (B) and in control, *Phd1*, or *Phd2* shRNA expressing RAW264.7 cells (D). E, The luciferase activity of HRE-luciferase vector after 24 hours of cotransfection with CA-HIF-1α expression vector or empty vector was measured. n=3. F, The *Tnf-α* gene promoter-luciferase activity after 24 hours of CA-HIF-1α vector or empty vector introduction followed by 6 or 24 hours of 100 ng/mL of LPS stimulation was measured. n=3 to 4. NS indicates not statistically significant, *P<0.05, **P<0.01 vs LPS (−) or control, ##P<0.01.

expression vector[21] was transiently introduced and a luciferase activity was measured. DMOG treatment for 24 hours strongly increased the HRE-dependent transcriptional activity (Figure 3B).

We also determined the levels of HIF-α in *Phd1*- or *Phd2*-depleted cells. Introduction of *Phd2* shRNA, but not *Phd1* shRNA, induced HIF-1α accumulation, whereas HIF-2α was not induced by either *Phd1* or *Phd2* shRNA (Figure 3C). HRE-dependent transcriptional activity was only increased in *Phd2*-depleted cells (Figure 3D).

A HIF-1α Overexpression Failed to Suppress the LPS-Induced *Tnf-α* Promoter Activation

To test whether accumulated HIF-1α by DMOG is responsible for the suppression of LPS-induced TNF-α induction, we determined the effect of overexpression of CA-HIF-1α.[22] The expression of CA-HIF-1α strongly increased HRE-dependent transcriptional activity (Figure 3E). However, *Tnf-α* gene transcriptional activity was not suppressed in CA-HIF-1α–expressing cells after 6 hours or 24 hours of LPS stimulation (Figure 3F).

DMOG Suppressed LPS-Induced *Tnf-α* Upregulation in *Hif-1α*–Depleted Cells

We next examined whether DMOG would be able to suppress the LPS-induced *Tnf-α* upregulation in the absence of HIF-1α. shRNA specific for *Hif-1α* gene strongly decreased the *Hif-1α* mRNA level and the DMOG-induced HIF-1α accumulation (Figure 4A and 4B). Then, *Hif-1α*–depleted cells were pretreated with DMOG for 1 hour and stimulated with 100 ng/mL of LPS for 4 hours. Consistent with a previous report,[12] the induction of *Tnf-α* mRNA was significantly

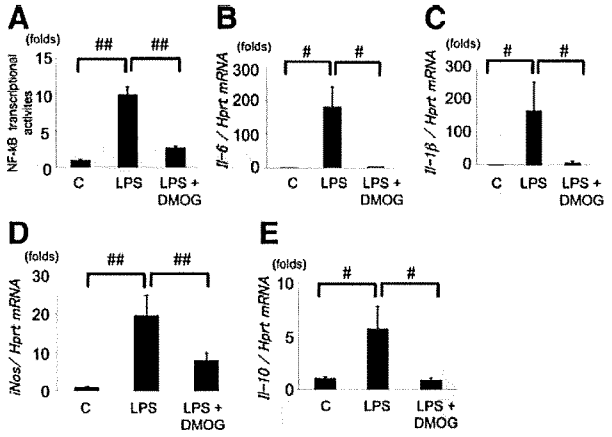

Figure 5. DMOG treatment suppressed LPS-induced NF-κB transcriptional activation and upregulation of other cytokines. A, LPS-induced NF-κB transcriptional activity after 8 hours of LPS treatment with pretreatment of 1 mmol/L of DMOG or DMSO for 1 hour was measured as luciferase activity. n=3. B through E, RAW264.7 macrophages were stimulated with 100 ng/mL of LPS for 4 hours with DMOG or DMSO pretreatment for 1 hour. The expressions of *Il-6* (B), *Il-1β* (C), *iNos* (D), and *Il-10* (E) were determined by real-time qPCR and normalized with the expression level of *Hprt* gene. n=4. #$P<0.05$, ##$P<0.01$.

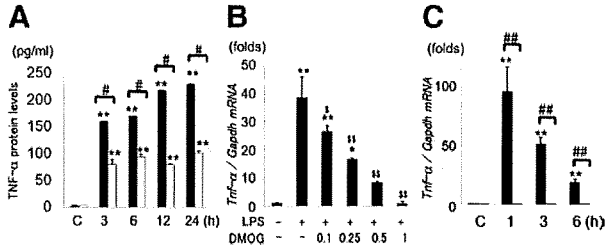

Figure 6. DMOG suppressed LPS-induced TNF-α upregulation in murine peritoneal macrophages and human THP-1 macrophages. A, Peritoneal macrophages from normal mice were stimulated with 100 ng/mL of LPS for varying periods indicated in the figure with pretreatment of 1 mmol/L of DMOG (open bar) or a vehicle DMSO (filled bar) for 1 hour. TNF-α concentration in the supernatant was determined by ELISA. n=3 to 4. B, Human THP-1 macrophages were stimulated with 100 ng/mL of LPS for 1 hour after pretreatment with varying concentrations of DMOG for 1 hour. *Tnf-α* mRNA level was determined by real-time qPCR and normalized with *Gapdh* mRNA levels. C, THP-1 cells were stimulated with 100 ng/mL of LPS for varying periods indicated in the figure with pretreatment of 1 mmol/L of DMOG (open bar) or a vehicle DMSO (filled bar) for 1 hour. #$P<0.05$, ##$P<0.01$, **$P<0.01$ vs control, $$P<0.05$, $$$P<0.01$ vs LPS.

reduced in *Hif-1α*–depleted cells (Figure 4C). However, DMOG further suppressed the LPS-induced *Tnf-α* upregulation in *Hif-1α*–depleted cells (Figure 4C and supplemental Figure V).

DMOG Treatment Suppressed LPS-Induced NF-κB Transcriptional Activation

Because both activation of NF-κB and mitogen-activated protein kinases (MAP kinases) is responsible for the LPS-induced TNF-α induction,[23] we examined whether DMOG would suppress an activation of MAP kinases such as p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK). Phosphorylation of these kinases, a surrogate marker of kinase activation, was strongly induced by 100 ng/mL of LPS but the activation was not reduced by DMOG pretreatment (supplemental Figure VIA through VIC).

Next, LPS-induced activation of NF-κB transcriptional activity was determined with NF-κB–dependent luciferase activity. LPS treatment for 8 hours strongly increased NF-κB transcriptional activity and DMOG pretreatment significantly suppressed the activation (Figure 5A). NF-κB nuclear translocation and binding capacity to NF-κB consensus site were determined by electrophoretic mobility shift assay and ELISA-based DNA-binding assay by using nuclear protein extract after LPS stimulation, respectively. However, translocation of NF-κB into the nucleus and binding capacity to NF-κB site was not decreased by DMOG pretreatment (supplemental Figure VIIA and VIIB).

The Effect of DMOG on Other Cytokine Productions

We examined the effect of DMOG on LPS-induced expression of other genes encoding inducible nitric oxide synthase (iNOS), proinflammatory cytokines (eg, interleukin [IL]-6, IL-1β) and antiinflammatory cytokine (eg, IL-10), of which expression is dependent on NF-κB. DMOG significantly suppressed LPS-induced upregulation of these genes (Figure 5B through 5E). Because all cytokines studied were suppressed by DMOG, we excluded the possible cytotoxic effect of DMOG. Flow cytometry to detect PI-positive dead cells revealed that 1 mmol/L of DMOG treatment for 24 hours did not affect cell viability in RAW264.7 macrophages (supplemental Figure VIII). The cytotoxic effect of DMOG was further ruled out by the evidence that DMOG upregulated *Vegf* gene expression (supplemental Figure IX).

DMOG Suppressed LPS-Induced TNFα Upregulation in Resident Peritoneal Macrophages and Human THP-1

Finally, to generalize the effect of DMOG on LPS-induced TNF-α upregulation, we analyzed the effect of DMOG on 2 different types of macrophages. One is murine peritoneal macrophages from normal mice, and the other is human monocyte cell line THP-1. Consistent with the results of RAW264.7 macrophages, DMOG pretreatment significantly suppressed LPS-induced TNF-α secretion in peritoneal macrophages (Figure 6A). DMOG also time- and dose-dependently suppressed LPS-induced *Tnf-α* mRNA upregulation in differentiated THP-1 macrophages (Figure 6B and 6C).

Discussion

In this article, we demonstrated that PHD inhibition by DMOG significantly suppressed LPS-induced expression of several proinflammatory genes encoding not only TNF-α but IL-6, IL-1β, iNOS, and antiinflammatory gene IL-10 in macrophages. Although DMOG treatment apparently raised HIF-1α level, the increased HIF-1α was not responsible for the suppression. And PHD1 among three PHD isoforms may be mainly responsible for the suppressive effect of DMOG on LPS function. These data indicated that PHD inhibition decreased cellular sensitivity to inflammatory stimuli and may have a therapeutic implication.

How does PHD inhibition suppress LPS-induced TNF-α upregulation? Because PHD is a negative regulator for HIF-1α or HIF-2α expression, one would expect that increased HIF-α might be responsible for the suppression. HIF-2α was undetectable in RAW264.7 macrophages, excluding the possible involvement of HIF-2α. In contrast, DMOG strongly induced HIF-1α accumulation and activated HRE-dependent transcription. However, DMOG suppressed LPS-induced *Tnf-α* upregulation even in *Hif-1α*–depleted cells. Moreover, *Phd1* knockdown significantly inhibited LPS-elicited TNF-α upregulation but did not increase HIF-1α levels. In addition, forced expression of stable form of HIF-1α (CA-HIF-1α) failed to inhibit TNF-α promoter activity. These evidences consistently indicate that DMOG-mediated suppression of the LPS effect does not depend on HIF-1α as well as HIF-2α.

The mechanism by which PHD inhibition attenuated LPS-induced TNF-α production is not clear at this point, but several possibilities may be considered. First of all, because NF-κB activation is an essential step for the induction of cytokines such as TNF-α, IL-6, IL-1β, iNOS, and antiinflammatory IL-10,[24,25] DMOG-induced NF-κB suppression may be a potential mechanism. We observed that DMOG did not suppress nuclear translocation or binding capacity to NF-κB consensus site but reduced NF-κB–dependent transcriptional activity. The mechanism by which DMOG suppressed NF-κB activation remains elusive. However, recent studies suggest that phosphorylation of NF-κB on Ser536 phosphorylation is essential for the NF-κB transcriptional activation.[26] Thus, DMOG may affect the phosphorylation of NF-κB to suppress LPS-induced transcriptional activation.

In contrast to our data, a previous report suggest that DMOG may enhance inflammatory reaction. Cummins et al described that PHD-induced hydroxylation of IκB kinase-β (IKKβ), an activator of NF-κB pathway, attenuated its kinase activity.[13] DMOG activates NF-κB pathway and induces proinflammatory cyclooxygenase 2 expression in HeLa cells.[13] The reason for the discrepancy between their study and ours is not immediately clear, but it may be possible that PHD inhibition causes different effects on different cell type, which is most likely reflecting differential expression pattern of PHD isoforms.[19,27] Alternatively, DMOG may increase the basal expression of proinflammatory genes[13] while decreasing the induction of these genes on inflammatory stimuli. Therefore, further study is needed to clarify the effect of PHD inhibition in several different experimental conditions of inflammation.

Other possible mechanisms for DMOG-elicited suppression of LPS-induced TNF-α upregulation may be the suppression of oxidative phosphorylation and global energy consumption. DMOG treatment significantly inhibits electron transport chain activity during mitochondrial respiration, leading to the reduced ATP production in cardiomyocytes.[28] DMOG also inhibits intracellular ATP consumption, an example of which is the reduction of contraction in cardiomyocytes.[28] Therefore, DMOG may suppress energy metabolism, leading to attenuation of inflammatory responses in macrophages.

Our isoform-specific knockdown experiments indicated that PHD1 was mainly responsible for LPS-induced TNF-α upregulation. In IKKβ hydroxylation, PHD1 is also mainly responsible.[13] PHD1 knockout mice caused reduced ATP production and consumption in skeletal muscle.[29] These data indicate that although PHD2 is generally important for HIF regulation,[30] PHD1 might have a distinct pathway rather than HIF to regulate various biological activities. Thus, further study is needed to identify a target molecule of hydroxylation by PHD1 to clarify the role of PHD1 in LPS-induced inflammation. In this study, we did not analyze the role of PHD3, because PHD3 was not expressed in RAW264.7 cells. However, PHD3 has a potential to compensate the role of PHD1 in other cells that express PHD3.[31] Thus, we cannot exclude the possible involvement of PHD3 in other inflammatory models in which PHD3 is present substantially.

In general, hypoxia is considered to induce or augment inflammatory responses. For instance, hypoxia augments LPS-induced TNF-α and iNOS expression in several cell lines including RAW264.7 macrophages and murine dendritic cells.[32–34] Thus, it may be counterintuitive that PHD inhibition suppresses LPS-induced TNF-α expression, because both hypoxia and PHD inhibition induce HIF-α accumulation and upregulation of HIF target gene expression. However, the biological effects caused by hypoxia or PHD inhibition are not necessarily the same or even opposite.[29] One example is a production of reactive oxygen species (ROS); hypoxia increases ROS production, whereas PHD inhibition decreases ROS.[29] If hypoxia-induced ROS production potentiates inflammation, reduced ROS production by PHD inhibition may attenuate inflammation. Thus, it is possible that hypoxia and PHD inhibition induce opposite biological responses in some cases. Our study indicates that PHD inhibition suppresses LPS-induced TNF-α upregulation, which is usually augmented by hypoxic exposure.[32,33]

Taken together, we provided the first-line evidence that PHD inhibition suppressed LPS-induced proinflammatory TNF-α production independently of HIF-1α in macrophages. TNF-α is involved in various pathological conditions, including sepsis, autoimmune disorders, atherosclerosis, and obesity-associated insulin resistance.[12,35,36] Antagonizing TNF-α has been shown to be protective for several inflammatory diseases.[37] Therefore, PHD inhibition might be a novel strategy for the treatment of inflammatory diseases.

Acknowledgments

The authors would like to acknowledge the technical expertise of the Support Center for Education and Research, Kyushu University.

Sources of Funding

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (21790731) and Japan Heart Foundation/ Novartis Grant for Research Award on Molecular and Cellular Cardiology (2008) to K.T.

Disclosures

None.

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Journal of Cardiology (2009) 53, 341–348

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Official Journal of the Japanese College of Cardiology
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ORIGINAL ARTICLE

Atorvastatin might improve ventricular electrostability and decelerate the deterioration of renal function in patients with heart failure and diabetes mellitus

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Received 22 September 2008; received in revised form 9 November 2008; accepted 3 December 2008
Available online 6 February 2009

KEYWORDS
Atorvastatin;
Heart failure;
Diabetes mellitus

Summary
Background and purpose: Previous studies suggested that statins have pleiotropic effects, such as improvements in endothelial function, as well as anti-inflammatory, anti-proliferative, and anti-oxidative effects. These effects might benefit patients with heart failure. In those patients, statins relieved symptoms, decreased the frequency of hospitalization, suppressed neurohumoral activation, and improved cardiac function. However, it remains unknown how statins impact pathophysiology of heart failure with diabetes mellitus. The aim of this study was to investigate the effects of atorvastatin on pathophysiology of heart failure with diabetes mellitus.
Methods and results: We enrolled retrospectively 128 patients with heart failure with diabetes mellitus who were admitted from January 2003 to December 2005. Among these patients, 80 received atorvastatin (statin group) and the remaining patients served as controls (non-statin group). At study entry, there were no significant differences in the patient profiles between the two groups except for the low-density lipoprotein cholesterol level being higher in the statin group. After the follow-up period of two years, the frequency of re-hospitalization, brain natriuretic peptide, premature ventricular contractions, Lown grade, and deterioration of glomerular filtration rate were significantly less in the statin group.

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doi:10.1016/j.jjcc.2008.12.002

Conclusion: Atorvastatin might benefit patients with heart failure and diabetes mellitus by improving ventricular electrical stability and decelerating deterioration of renal function.

Introduction

Previous randomized clinical trials have shown that statins reduce cardiovascular events in patients with and/or without coronary artery disease. Statins have been shown to improve endothelial function [1], decrease plasma levels of proinflammatory cytokines [2], and exert antihypertrophic [3,4], antioxidant [5], and antifibrotic [6] effects on myocardium. Furthermore, other reports suggested that statins have beneficial effects on immune function, macrophage metabolism, and cell proliferation irrespective of changes in low-density lipoprotein (LDL) cholesterol concentrations [7].

Recent clinical trials suggested that statins might benefit patients with heart failure [8–13]. Short-term statin therapy improved cardiac function, neurohumoral imbalance, and symptoms in patients with idiopathic dilated cardiomyopathy [8]. Long-term atorvastatin therapy suppressed neurohumoral activation and improved cardiac function in mild to moderate heart failure [9]. Compared with a lower dose, high-dose treatment with atorvastatin in patients with stable coronary disease significantly reduced hospitalization for heart failure [10]. However, it remains unknown how statins impact pathophysiology of heart failure with diabetes mellitus, which is known to have deleterious effects on heart failure [14,15] and coronary artery disease [16].

The aim of this study was to investigate the effects of atorvastatin on pathophysiology of heart failure with diabetes mellitus. The results indicated that atorvastatin might benefit heart failure with diabetes mellitus by improving ventricular electrostability and decelerating the deterioration of renal function.

Subjects and methods

Patient populations

We retrospectively studied patients with symptomatic acute heart failure and diabetes mellitus who were admitted to Aso Iizuka Hospital from January 2003 to December 2005. The criteria for enrollment in the study were the clinical evidence of acute heart failure diagnosed by Framingham criteria [17], and diabetes mellitus diagnosed by the guideline of diabetes mellitus of the Japan Diabetes Society. In those patients, the New York Heart Association (NYHA) functional classification on admission ranged between II and IV. We excluded chronic obstructive pulmonary disease, right heart failure, and patients who had already taken atorvastatin or other statins. All patients were treated for acute heart failure, and were discharged after the improvement of heart failure. We enrolled 128 patients with heart failure and diabetes mellitus. Among them, 80 patients started to receive atorvastatin (10 mg) and the remaining 48 patients did not receive any statins (non-statin group). We followed up both groups for two years. As biochemical biomarkers, we measured plasma brain natriuretic peptide (BNP) and hemoglobin A1c (HbA1c). We calculated estimate of glomerular filtration rate (eGFR) from serum creatinine value and age using Japanese-coefficient-modified MDRD study [18]. As physiological biomarkers, we conducted echocardiography and 24-h Holter monitoring, and the severity of ventricular arrhythmias was evaluated in terms of Lown grade [grade 0: no premature ventricular contraction (PVC); grade I: <30 PVC/h, grade II: >30 PVC/h; grade III: multiform PVC; grade IVa: couplets; grade IVb; ventricular tachycardia runs] [19]. We acquired those biomarkers just before the statin therapy in the statin group and before discharge in the non-statin group, and one and two years after the discharge in both group. Hospitalizations due to worsening heart failure were diagnosed by the Framingham criteria, as described above.

Statistical analysis

Normally distributed variables were expressed as mean ± S.D. Unpaired t test or Mann–Whitney U test was used to compare the differences in normally distributed variables, respectively, between the statin and non-statin groups. The rate of re-hospitalization due to worsening heart failure between the statin and non-statin groups was compared by Kaplan–Meier analysis. All statistical tests were carried out against the baseline characteristics. Differences were considered significant at a p value of <0.05.

Table 1 Patient characteristics.

	Statin	Non-statin	*P*-value
n	80	48	NS
Male/female	54/26	34/14	NS
Age	65 ± 7	61 ± 5	NS
BMI	22 ± 4	23 ± 3	NS
Current smoker	18 (23%)	10 (20%)	<0.05
Causes of heart failure			
Coronary artery disease	22 (27%)	9 (18%)	NS
Dilated cardiomyopathy	25 (32%)	13 (28%)	NS
Hypertensive heart disease	14 (18%)	10 (21%)	NS
Valvular heart disease	15 (19%)	11 (23%)	NS
Systolic blood pressure (mmHg)	130 ± 17	128 ± 14	NS
Diastolic blood pressure (mmHg)	72 ± 14	68 ± 9	NS
Heart rate (bpm)	82 ± 9	79 ± 5	NS
Medications			
Diuretics	74 (92%)	44 (92%)	NS
β-Blockers	53 (66%)	34 (71%)	NS
ACE inhibitors	75 (94%)	46 (96%)	NS
Angiotensin receptor blocker	3 (4%)	2 (4%)	NS
Sulfonylurea	12 (15%)	8 (17%)	NS

Data are presented as number (%) or mean ± S.D. BMI, body mass index; bpm, beats per minute; ACE, angiotensin-converting enzyme.

Results

Patient characteristics at baseline

The patient profiles at enrollment are summarized in Tables 1 and 2. As can be seen in Table 1, there were no significant differences in age, gender, or the prevalence of dilated cardiomyopathy, hypertensive heart disease, or valvular heart disease between the non-statin group and statin group just before statin therapy. Medications did not differ either. The frequency of patients taking

Table 2 Patient characteristics (2).

	Statin	Non-statin	*P*-value
Total cholesterol (mg/dl)	227 ± 17	200 ± 10	0.042
LDL cholesterol (mg/dl)	156 ± 11	122 ± 13	0.008
HDL cholesterol (mg/dl)	44 ± 8	47 ± 7	NS
Triglycerides (mg/dl)	129 ± 7	132 ± 11	NS
FBS (mg/dl)	112 ± 7	119 ± 5	NS
HbA1c (%)	6.6 ± 0.7	6.3 ± 0.4	NS
BNP (pg/ml)	128 ± 27	142 ± 36	NS
eGFR (ml/min/1.73 m^2)	62.4 ± 7.9	66.8 ± 4.4	NS
LVEF (%)	35 ± 7	33 ± 5	NS
LVEDD (mm)	56 ± 5	57 ± 7	NS
LVESD (mm)	40 ± 4	42 ± 7	NS
PVC per 24 h	1288 ± 362	1194 ± 443	NS
Lown grade			
I	28 (35%)	15 (31%)	NS
II	43 (54%)	26 (54%)	NS
III	8 (10%)	6 (13%)	NS
IVa/IVb	1 (1%)/0 (0%)	1 (2%)/0 (0%)	NS/NS

Data are presented as mean ± S.D. LDL, low-density lipoprotein; HDL, high-density lipoprotein; FBS, fasting blood glucose; HbA1c, hemoglobin A1c; BNP, brain natriuretic peptide; eGFR, creatinine-based estimate of glomerular filtration rate; LVEF, left ventricular ejection fraction; LVDDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; PVC, premature ventricular contraction.

angiotensin receptor blockers or oral hypoglycemic agents also did not differ between the non-statin and statin groups. All patients with hypoglycemic agents took sulfonylurea. There is no patient with insulin-therapy for diabetes mellitus in the present study. As anticipated, the statin group had a higher prevalence of coronary artery disease than the non-statin group.

As shown in Table 2, fasting blood glucose (FBG), HbA1c, BNP, or eGFR did not differ between the two groups. As anticipated, however, total cholesterol and LDL cholesterol were higher in the statin group than in the non-statin group. Left ventricular ejection fraction (LVEF), left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), the frequency of PVC and the frequency of patients who had Lown grade I, II, III, or IV did not differ between the two groups. There is no patient with Lown grade 0 in the present study.

Effects of atorvastatin on biochemical and physiological biomarkers

Effects of atorvastatin on biochemical biomarkers are summarized in Table 3. Atorvastatin markedly decreased total cholesterol and LDL cholesterol. As a result, LDL cholesterol values at the follow-up period of one year were comparable between the two groups and got lower at two years in the statin group than the non-statin group. BNP was lower at one year and got lower at two years in the statin group than the non-statin group. Systolic blood pressure, LVEF, LVEDD, LVESD, and HbA1c remained not different between the two groups.

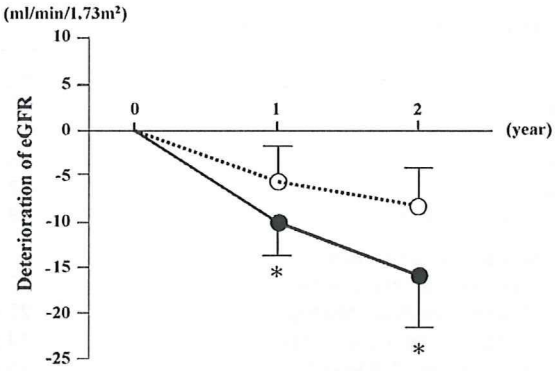

Figure 1 Changes in eGFR at entry, one and two years. Open circle and solid line indicate statin group. Closed circle and dotted line indicate non-statin group. *$p < 0.05$. eGFR, creatinine-based estimate of glomerular filtration rate.

As shown in Fig. 1, eGFR decreased more in the non-statin group than in the statin-group (-10.6 ± 2.8 ml/min/1.73 m^2 vs. -6.1 ± 3.3 ml/min/1.73

Table 3 Changes in LDL-C, BNP, LVEF, LV dimension, systolic blood pressure, and HbA1c at one and two years.

	Statin	Non-statin	*P*-value
LDL-C (mg/dl)			
1 year	98 ± 16	116 ± 13	NS
2 years	94 ± 9	112 ± 11	<0.05
BNP (pg/ml)			
1 year	101 ± 12	136 ± 13	<0.05
2 years	76 ± 11	132 ± 13	<0.05
LVEF (%)			
1 year	38 ± 6	35 ± 6	NS
2 years	40 ± 7	38 ± 7	NS
LVEDD/LVESD (mm)			
1 year	57 ± 6/41 ± 4	59 ± 4/44 ± 5	NS/NS
2 years	58 ± 4/42 ± 4	60 ± 7/46 ± 7	NS/NS
Systolic blood pressure (mmHg)			
1 year	118 ± 13	122 ± 15	NS
2 years	116 ± 11	120 ± 13	NS
HbA1c (%)			
1 year	6.4 ± 0.3	6.5 ± 0.4	NS
2 years	6.1 ± 0.4	6.5 ± 0.3	NS

Data are presented as mean ± S.D. LDL-C, low-density lipoprotein cholesterol; BNP, brain natriuretic peptide; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; HbA1c, hemoglobin A1c.

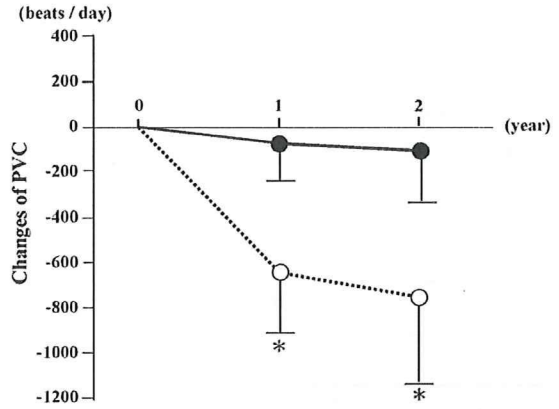

Figure 2 Changes in PVCs at entry, one, and two years. Open circle and solid line indicate statin group. Closed circle and dotted line indicate non-statin group. *$p < 0.05$. PVC, premature ventricular contraction.

m^2, $p < 0.05$) at one year. The difference increased at two years (-16.8 ± 4.1 ml/min/1.73 m^2 vs. -8.3 ± 5.4 ml/min/1.73 m^2, $p < 0.05$). Atorvastatin might have decelerated deterioration of renal function, and thereby might have a potent protective effect on renal function.

Shown in Fig. 2 is the effect of atorvastatin on the frequency of PVCs. Atorvastatin markedly decreased PVCs at one year and two years. There were no changes in PVCs in the non-statin group. Furthermore, the frequency of patients who had Lown grade ≥II was significantly lower in the statin group than in the non-statin group at one year and two years (Fig. 3).

Table 4 Cardiovascular events, hospitalization, and mortality at one and two years.

	Statin	Non-statin	*P*-value
Cardiovascular events			
1 year	5 (6%)	4 (8%)	NS
2 years	8 (10%)	6 (13%)	NS
CHF with hospitalization			
1 year	7 (9%)	8 (17%)	0.003
2 years	16 (20%)	15 (31%)	0.005
All-cause mortality			
1 year	0	0	NS
2 years	0	1	NS

Data are presented as number (%) or mean ± S.D. CHF, congestive heart failure.

Cardiovascular events and hospitalization

No patient died in the statin group, whereas one patient suddenly died in the non-statin group. Treatment with atorvastatin did not significantly reduce cardiovascular events, which was defined as nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization, or cardiovascular death (Table 4). However, the frequency of re-hospitalization due to worsening heart failure was significantly reduced in the statin group than in the non-statin group, determined by Kaplan–Meier analysis (Fig. 4). For the patients with atorvastatin, the hazard ratio for re-hospitalization due to worsening heart failure was 0.68 (95% CI, 0.51–0.84) (Fig. 4).

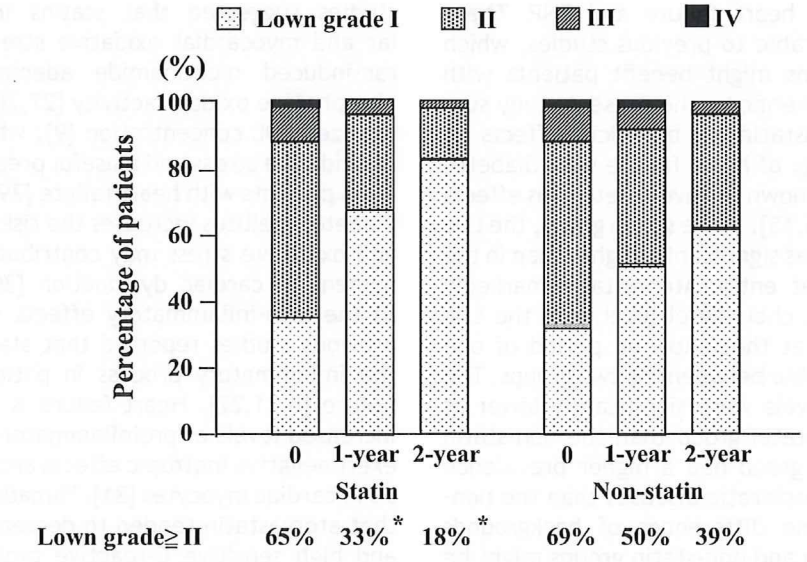

Figure 3 Kaplan–Meier analysis estimates for re-hospitalization due to heart failure. Solid line indicates non-statin group, and dotted line indicates statin-group. *$p < 0.05$.

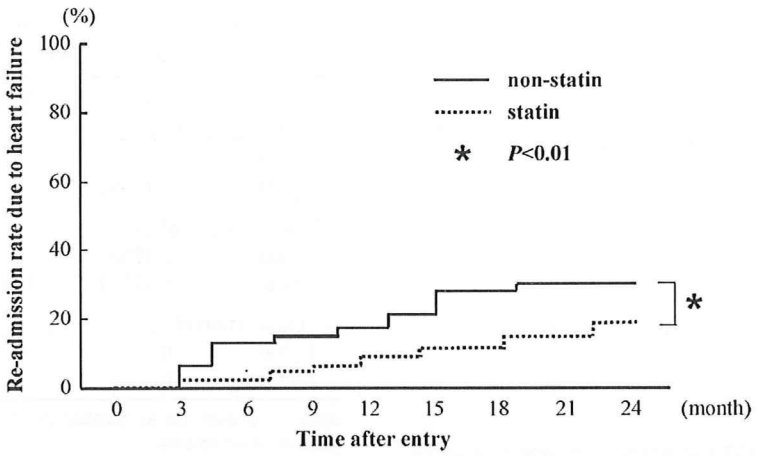

Figure 4 Lown grades at entry, one year, and two years after (grade 0: no PVC; grade I: <30 PVC/h, grade II: >30 PVC/h; grade III: multiform PVC; grade IV: couplets and/or ventricular tachycardia runs). *$p < 0.05$.

Discussion

In the present study, we demonstrated that atorvastatin reduced the frequency of re-hospitalization due to worsening heart failure, BNP, the frequency of PVCs, Lown grade and decelerated deterioration of GFR in the patients with heart failure and diabetes mellitus. These results suggested that atorvastatin might benefit patients with heart failure and diabetes mellitus by improving ventricular electrostability and decelerating deterioration of renal function.

Recent clinical trials and basic studies suggested that statins might benefit patients with heart failure [8–13,20–24]. In the present study, atorvastatin reduced the frequency of re-hospitalization due to worsening heart failure and BNP. These results are comparable to previous studies, which showed that statins might benefit patients with heart failure. Furthermore, the present study suggested that atorvastatin has beneficial effects on the pathophysiology of heart failure with diabetes mellitus, which is known to have deleterious effects on heart failure [14,15]. In the statin group, the LDL cholesterol level was significantly higher than in the non-statin group at entry. Atorvastatin markedly decreased the LDL cholesterol level, and the LDL cholesterol levels at the follow-up period of one year were comparable between the two groups. The LDL cholesterol levels were significantly lower at two years in the statin group than the non-statin group. The statin group had a higher prevalence of coronary atherosclerotic diseases than the non-statin group. These differences of backgrounds between the statin and non-statin groups might be responsible for the results in this study. However, these results indicate that the risks for atherosclerosis and ischemic heart diseases were higher in the statin group than in the non-statin group. Despite those with a background predisposed to coronary heart disease, atorvastatin significantly benefits the patients with heart failure. It is conceivable that atorvastatin might be a novel strategy of treatment for heart failure and diabetes mellitus.

The mechanism by which atorvastatin benefits patients with heart failure and diabetes mellitus remains unknown. We conjecture three mechanisms: anti-oxidant, anti-inflammatory, and improvement of the sympatho-vagal balance. In patients with heart failure, increased oxidative stress is associated with reduced LV function and severity of heart failure [25,26]. Previous studies suggested that statins inhibited vascular and myocardial oxidative stress by inhibiting rac-induced nicotinamide adenine dinucleotide phosphatase oxidase activity [27,28], and reducing oxidized LDL concentration [9], which is a marker of oxidative stress and a useful predictor of mortality in patients with heart failure [29]. Furthermore, diabetes mellitus increases the risk of heart failure and oxidative stress may contribute to the development of cardiac dysfunction [30]. With regard to the anti-inflammatory effects of atorvastatin, previous studies reported that statins suppressed the inflammatory process in patients with heart failure [8,21,22]. Heart failure is associated with increased levels of proinflammatory cytokines that exert negative inotropic effects and induce apoptosis in cardiac myocytes [31]. Yamada et al. reported that atorvastatin tended to decrease interleukin 6 and high sensitive C-reactive protein in patients with heart failure [9]. Finally, we consider that atorvastatin might improve the imbalance between

the sympathetic and parasympathetic nerve activity and this improvement resulted in the reduction of the frequency of PVCs. A previous study reported that statin therapy restored sympatho-vagal balance in experimental heart failure [23]. We have demonstrated that atorvastatin reduced the oxidative stress in the cardiovascular center of the brainstem [32], in which oxidative stress increased sympathetic nerve activity in hypertensive animal models [33]. These reports suggest that atorvastatin might reduce the oxidative stress in the cardiovascular center, which, in turn, decreases the sympathetic nerve activity, the frequency of PVCs, and Lown grade. Further investigations are needed to clarify the mechanisms.

Renal dysfunction has been known to worsen heart failure [34]. Campese and Park suggested that statin-mediated alterations in inflammatory responses and endothelial function reduced proteinuria and the rate of progression of kidney disease [35]. In the present study, atorvastatin protected the progressive worsening in renal function for two years in the patients with heart failure and diabetes mellitus. These results suggest that atorvastatin might prevent the worsening of heart failure through a renoprotective effect. Additional prospective and randomized trials in the Japanese population are needed to determine whether atorvastatin is truly renoprotective.

Limitations

There are several limitations to the present study. First, the study was retrospective, and observational. The number of patients enrolled is also limited. Second, we were not able to determine whether the beneficial effect of atorvastatin on heart failure with diabetes mellitus is a class effect or not. The results of the present study should be validated by large, prospective, well-controlled, and randomized clinical trials. Third, we did not measure the activity of sympathetic nerve activity, parasympathetic nerve activity, and sympatho-vagal balance using variability of R–R interval and blood pressure analysis. In the present study, we are not able to suggest that the data indicate the improvement of the imbalance between sympathetic and parasympathetic nerve activity by atorvastatin in heart failure with diabetes mellitus.

Conclusion

Atorvastatin might benefit patients with heart failure and diabetes by improving ventricular electrical stability and decelerating deterioration of renal function. Atorvastatin might be a novel strategy of treatment for heart failure and diabetes.

Acknowledgment

We are grateful to the staff in the cardiovascular center of Aso-Iizuka hospital.

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Clinical and Experimental Hypertension, 31:241–249, 2009

ISSN: 1064-1963 print / 1525-6006 online
DOI: 10.1080/10641960902822492

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Cilnidipine Inhibits the Sympathetic Nerve Activity and Improves Baroreflex Sensitivity in Patients with Hypertension

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N-type calcium channel blocker, cilnidipine, is reported not to increase the heart rate in spite of the strong depressor effect. However, it has not been determined whether cilnidipine has the sympatho-inhibitory effects or not. Moreover, the effect of cilnidipine on the baroreflex control has not been determined. The aim of this study was to determine the effect of cilnidipine on sympathetic and parasympathetic nerve activity, and baroreflex sensitivity. We studied five hypertensive patients treated with 10 mg cilnidipine (10-mg group) and five hypertensive patients treated with 20 mg cilnidipine (20-mg group). Before the treatment and 6 months after the treatment, we measured the blood pressure, spontaneous baroreflex sensitivity (BRS), heart rate variability (HRV), and blood pressure variability (BPV). After 6 months, systolic blood pressure (SBP) and the low-frequency component of systolic BPV expressed in normalized units (LFnuSBP), as the parameter of sympathetic nerve activity, was significantly decreased in both groups, and the suppressive effects were stronger in the 20-mg group than in the 10-mg group. The high-frequency component of HRV expressed in normalized units, as the parameter of parasympathetic nerve activity, and BRS were significantly increased in 20-mg group, but not significant in 10-mg group. These results suggest that 6 months treatment with cilnidipine for hypertension has the sympatho-inhibtory effect, and that high-dose cilnidipine improves the parasympathetic nerve activity and baroreflex control in patients with hypertension.

Keywords N-type calcium channels blocker, hypertension, sympathetic nerve activity, baroreflex sensitivity

Introduction

Hypertension is an established risk factor in the prognosis of cardiovascular diseases and organ damage. It may be feasible for patients with hypertension or at high cardiovascular risk to receive a blood pressure-lowering medication in order to achieve a

Submitted September 3, 2007; revised January 17, 2008; accepted April 24, 2008.
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